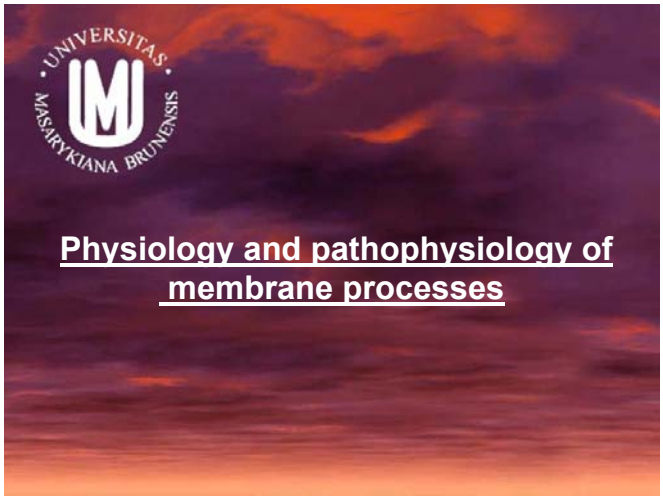
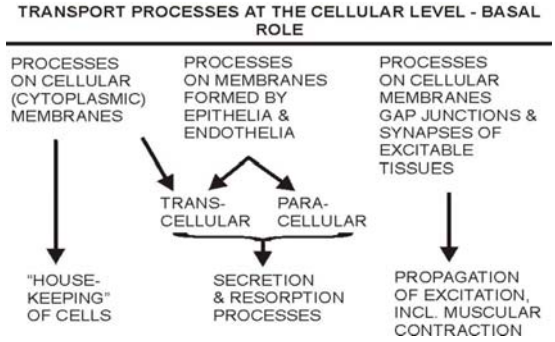


Physiology and pathophysiology of membrane processes

1. Physiology of membrane transports

1.1 General types of transports

Important: cellular pathology, kidney, gut, axcitable tissues
The basic purpose of transport processes at the cellular level (Fig. 1)
We look for: force, direction and factors („resistence")



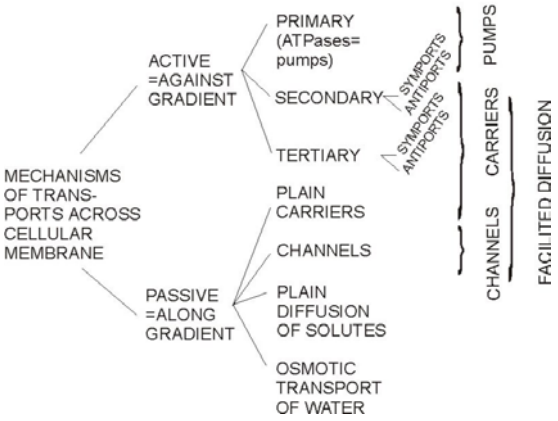
1. Bulk flow
Special instances:
Filtration across capillary wall: $V' = F * L * (\Delta P - \Delta\pi)$
Osmosis (Δc, $\Delta\pi$) → bulk flow across paracellular spaces and cytoplasmatic membranes
Bulk flow → solvent drag :
Flow of the solvent → ↑rate of movement of a solute (over diffusion)
Example: transfer of solutes across membranes by osmotically driven water (= bulk flow)

2. Diffusion = macroscopic flow of material from a region of high concentration to a region of lower concentration that results from the random Brownian motion of the molecules Ions: complicated by electric gradient – still „facilitated diffusion"
Diffusion flow ≈ permeability * Δc, i.e., linear relationship flow – concentration difference
Plain diffusion across cellular membranes:
Glycerol: no carrier, no charge, only Δc
Physiologically: water (mainly osmosis through carriers, however), O_2, CO_2, NH_3, ethanol, urea...
Not ions
Plain diffusion across paracellular shunts
No substantial difference between bulk flow and diffusion

3. Volume resorption paracellularily across the wall of resorptive epithelia:
Δc (small electrolytes), $\Delta\pi$. No hydrostatic pressure drive
Components: bulk flow (→ solvent drag) + diffusion

4. Facilitated diffusion
Transcellular flows take place mainly through specialized transmembrane proteins. Types of membrane transports – Fig. 2

KINDS OF TRANSPORTERS



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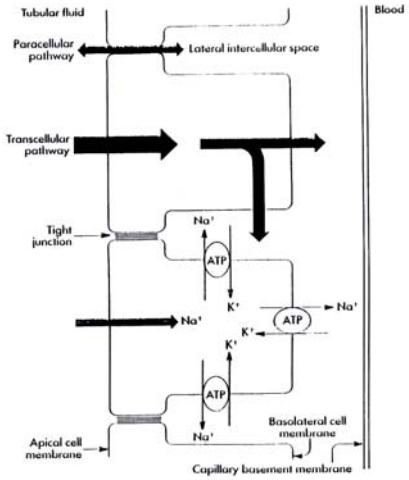
Paracellular flows in leaky epithelial and endothelial layers take place through s.c. tight junctions (Fig. 3 , Fig. 4). The preformed elements are incorporated into the lateral cell membrane → ↑number of cords → tight junction complex expands deeper into the intercellular gap; regulation of permeability of t.j.: ↑ glucose in bowels, ↓bacterial overgrowth in bowels



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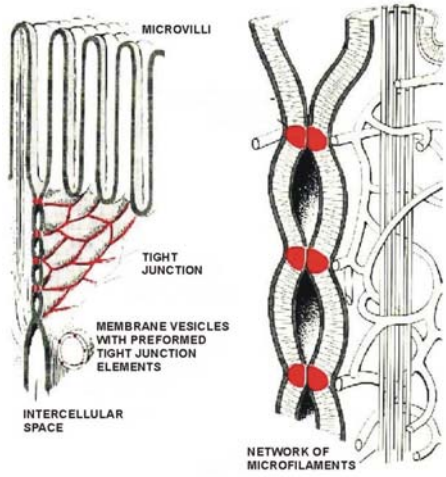

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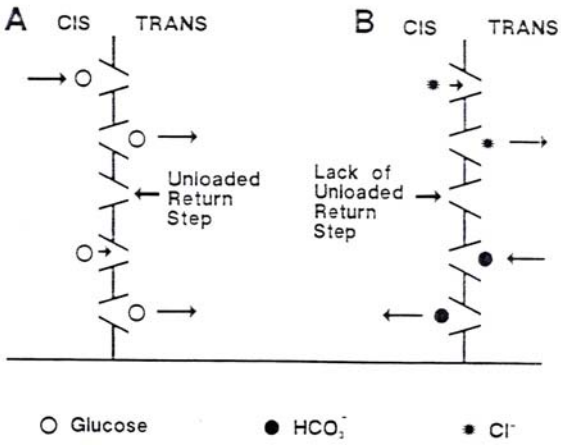

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Uniport: Small stiff tube. Erythrocytic glucose carrier - Fig. 5
- flow higher than with plain diffusion
- saturation kinetics
- competitive suppression

Antiport = countertransport → solute exchange, secondary active transport (if Na^+ is involved)
- Cl^-/HCO_3^- (erythrocytic)
- Na^+/Ca^{2+}
- Na^+/H^+

Symport
- Na+ and other metabolit (glucose, aminoacids) → secondary active transport
- NaCl/KCl symport

Ionic channels:

No⁺ and complex with ions → much higher velocity of transport
Channels do not determine direction and the steady state of the flow
Fig. 6: Various types of channel gating.
Gated channels → action potentials, non-gated channels → resting potentials
Common ions → blocking of channels
Channles for cations – selective; for anions – Cl^- only
Permeability of channels is a relatively constant property, but it cannot be ascertained easily. It used do be substituted by their conductivity, which is dependent, however, on the ionic concentrations on both sides of the channel

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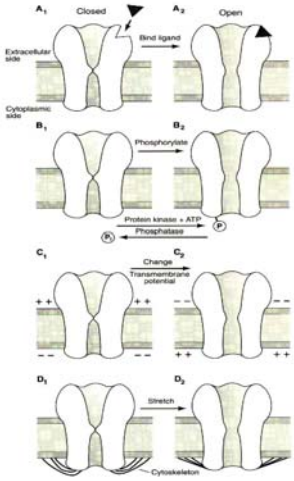

Saturation kinetics
Fig. 7:
Classification of ionic channels

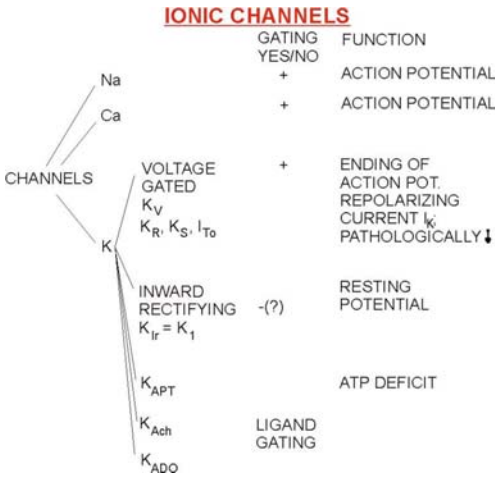

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Fig. 8:
Na^+ channel states. Generally: gated channels are allosteric proteins having two or more relative stable conformation states

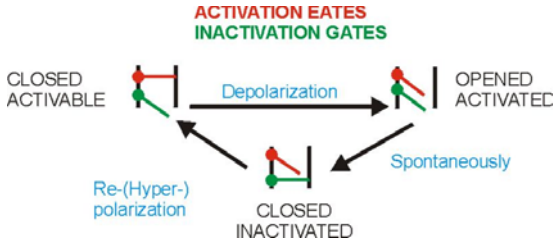

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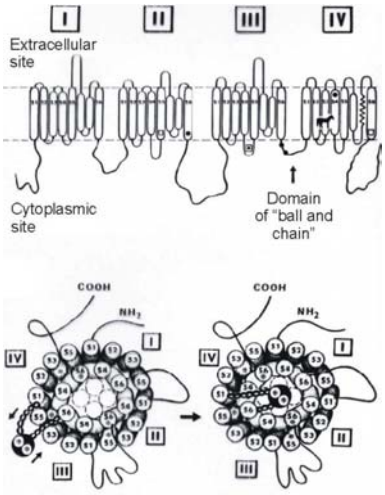
Fig. 9: Molecular structure of Na^+ channel
Channels are genetically determined (by several genes, as may be), have isoforms (gene polymorphism, splicing) → different speed of opening and closing, sensitivity to regulators



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Diffusion potentials „upon“ (across) the membrane

Fig. 10: The voltage is conditioned by a very small fraction of ions

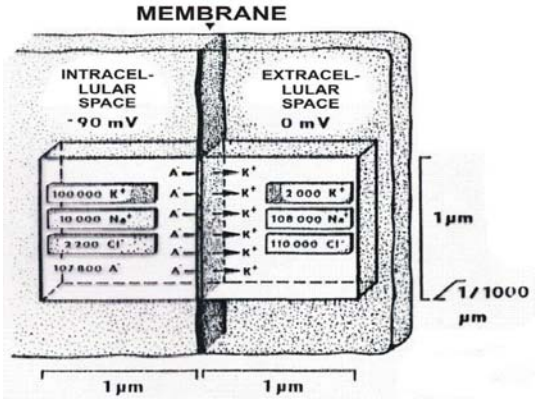
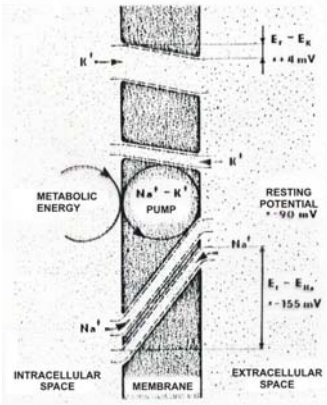


Fig. 11: Passive outflow of K^+ (through K_{ir} channels) → resting membrane potential. The outflow ceases when the electric force equals the concentration „force“ for the given ion – equilibrium (Nernst) potential for the ion



$$E_{ion} = -\frac{R*T}{z*F} * \log\frac{[Ion]_i}{[Ion]_o}$$

$[Ion]_i$ = intracellular concentration of ion X, [Ion]o = extracellular concentration of ion X, *R* = gas constant, *T* absolute temperature, *z* valence of the iont (negative for an aniont), *F* = constant of Faraday. Lumping the constants *R*, *T* and *F* together, we get a constant -61 mV.

For potassium:

$$E_K = -61\ mV \log_{10}\frac{[K^+]_i}{[K^+]_o}$$

If $[K^+]_i / [K^+]_o = 35$ (Fig. 12),
than E_K = -61 mV * log 35 = -61 mV * 1,59 = -94 mV.
Note: A few K^+ ions in high concentration gradient → membrane polarization
Massive potassion ions outflow, e.g., in hypoxia → ↓concentration gradient → loss of polarization (↓ membrane potential)

Fig. 12: Ionic concentrations, gradients and equilibrium potentials

Ion	Concentrations		Blood:Cytoplasma Gradient	Equilibrium voltage E_{ion}
	Blood	Cytoplasma		
Na^+	145 mmol	12 mmol	12:1	+66 mV
K^+	4 mmol	140 mmol	1:35	-94 mV
H^+	40 nmol	100 nmol	1:2,5	-24 mV
Cl^-	115 mmol	4 mmol	29:1	-89 mV
HCO_3^-	25 mmol	10 mmol	2,5:1	-24 mV
Mg^{2+}	1,5 mmol	0,8 mmol	1,9:1	+8 mV
Ca^{2+}	1,8 mmol	100 nmol	18000:1	+130 mV
HPO_4^{2-}	1 mmol	60 mmol		

Membrane potential produced by several ions
For more than one ion (Goldmans equation):

$$Er = \frac{RT}{F\,P_K} * \ln\frac{P_K[K^+]_o + P_{Na}[Na^+]_o + PCl[Cl^-]_i}{[K^+]_i + P_{Na}[Na^+]_i + PCl[Cl^-]_o}$$

The share of potassium is the highest

Driving force for a ion

Driving force for a ion = current membrane potential E_m - E_{ion}
Fig. 13 and 14 - examples

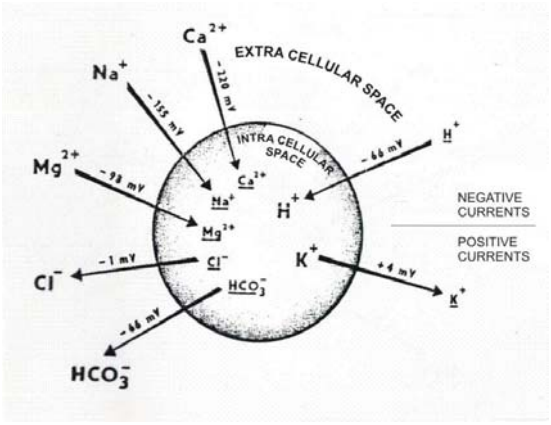
DRIVING FORCES FOR IONS

J = E * L * Z (> 0 kat, < 0 ani)
J: Current; E: Driving force; L: Conduct. (L > 0)

J has the sign: In cations - of E; In anions - opposite to E

Def: J: + Out, - Inward

Ion	Driving force E	Z (sign)	Driving force (sign)	Current
K^+	-90 - (-94) = +4	+	+	+(out)
Na^+	-90 - (+66) = -156	+	-	-(in)
H^+	-90 - (-24) = -66	+	-	-(in)
Ca^{2+}	-90 - (+130) = -220	+	-	-(in)
Cl^-	-90 - (-89) = -1	-	-	+(out)
HCO_3^-	-90 - (-24) = -66	-	-	+(out)



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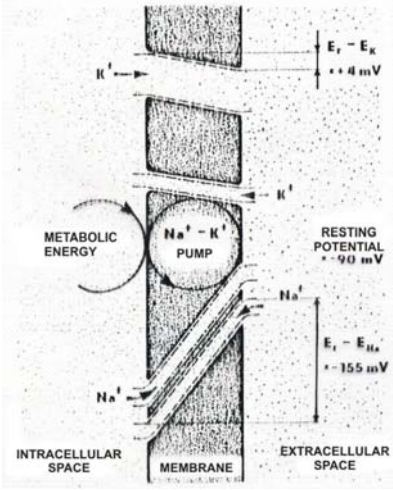
Primary active transport

Fig. 11 : Na/K - ATPase. The pump changes $3Na^+$ for $2K^+ \rightarrow$ electrogenicity $\rightarrow$ -10mV added to the diffusion potential. Negative feedback: the transport velocity rises with the concentration of transported ions
Na/K - ATPase is blocked by Ca^{2+} at the inner membrane surface
Maintains osmolality and volume of cells
Stimulation: trijodthyronin, aldosteron, β-adrenergic agonists



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Other transports

Fig. 15: membrane transports od Ca^{2+}

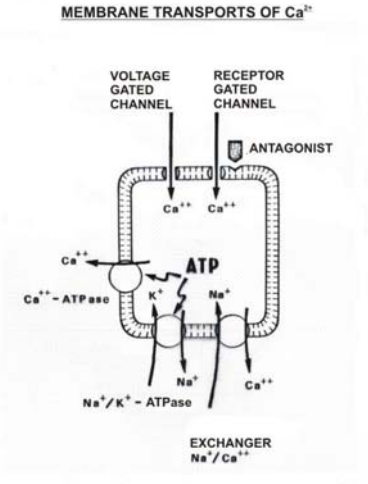

Fig. 16: Transport systems on a cellular membrane

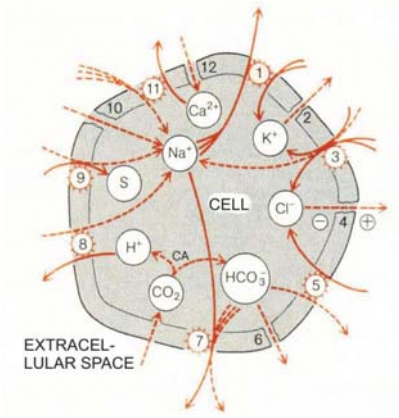

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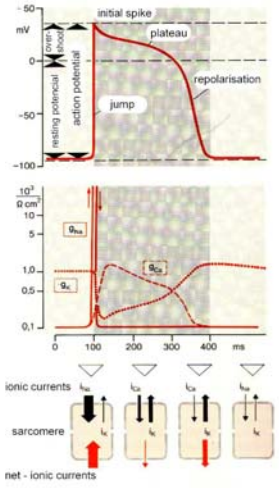
Fig. 17: Action potential in a myocardial sacromere



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Depolarization
→ opening of Na channels → massive Na influx → massive depolarization (phase 0)
Spontaneous inactivation of gated Na channels → slight repolarization (phase 1)
Plateau phase (2):
↓K conductivity → K cannot immediately repolarize
→ opening of DHP receptors → influx of Ca
→ opening of ryanodine receptors of SR → efflux of Ca from SR → contraction
→ prolonging depolarization

Repolarization (3):
↑Ca intracellular concentration
→ inactivation of DHP receptors (L type channels) → ↓Ca concentration → repolarization
→ opening of K channels → K efflux → repolarization

Diastolic phase (4): Na/Ca exchanger and (calmodulin →) CaATPase → expelling Ca out again

1.2 Membrane processes in epithelial tissues

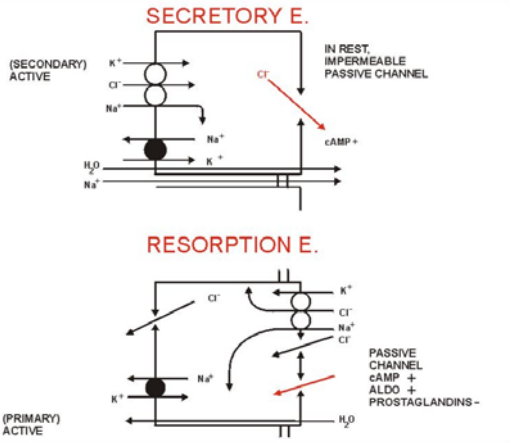
Epithelia in general

More complex situation than with a single cell surrounded by intercellular fluid:
- The epithelial cells are situated between intercellular fluid and resorbed/secreted fluids
- The permeability for the same ion may be different on both membranes

Consequences:
- Transepithelial electric gradient may arise which influences both transcellular and paracellular flows (e.g., renal tubuli)
- Driving forces for ions may be different on apical and (baso)lateral membranes

Anyway, the classical two-membrane model is still valid: both intracellular and transcellular mechanisms are the same

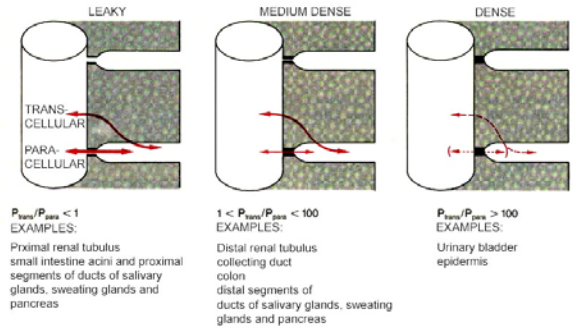
Resorption and secretory epithelia (Fig. 18)



Secretion epithelia: Cl^- is „pushed" by the cell in the lumen of a tubulus. The Na^+/K^+-ATPase maintains low intracellular Na^+, Cl^- penetrates into the cell by $NaCl \cdot KCl$ symport and is secreted into the lumen. Cl^- channel at the apical site (gated, e.g., by cAMP), closed in rest. Cl- secretion → secretion of Na and water

Resorption epithelia: Resorption of Na^+ is of basal importance. Na passage is passive on the apical site, through a channel which (or whose number) is regulated via cAMP by several hormones, incl. aldosterone. The Na passsage on the basolateral membrane is primarily active. This means sucking instead of pushing. If present, the Cl^- transporters are situated so that they accept Cl^- into the cell from the apical site

Leaky and tight epithelia (Fig. 19)
Division according to the proportion between para- and transcellular flows
Leaky epithelia: considerable volumes of transported water and solutes, e.g., volume resorption with resorption epithelia. (Leaky secretion epithelia: only acini and proximal segments of salivary and sweat glands)



Active transports – only through *cellular* membranes (pumps and symports). Only fully passive transports could take place paracellularly (via tight junctions in epithelia): diffusion and electrically driven movement of ions, osmotic stream of water, solvent drag – all according to some gradient. If paracellular flows are easily possible in leaky epithelia, the effect of active flows could be paralysed easily. It is not possible to maintain a high concentration gradient (the particles may return paracellularly), neither a large electric gradient is realizable then – this is proportional to the log of the ratio of the concentrations

Moderately tight epithelia are able to transport against gradients – the particles cannot return easily

Examples of the types of epithelia

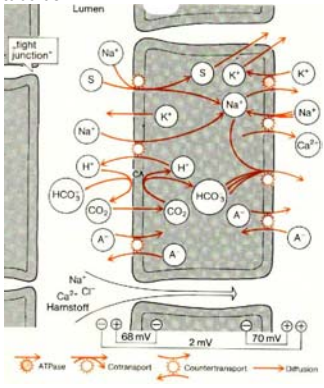
Fig. 20: Leaky and tight epithelia among secretory and resorptive epithelia

Types of epithelia according to the transport direction and according to the trans, and paracellular components

EPITHELIA	SECRETING	RESORBING
"LEAKY"	ACINI & PROXIMAL SEGMENTS OF THE DUCTI OF SWEAT & SALIVARY GLANDS &PANCREAS	RENAL PROXIMAL TUBULUS SMALL INTESTINE GALL BLADDER
SEMI-LEAKY		HENLE'S LOOP DIST. & COLLECTING TUBULI COLON, RECTUM DISTAL SEGMENTS OF THE DUCTI OF SWEAT & SALIVARY GLANDS & PANCREAS
TIGHT	BARRIERS: URINARY BLADDER EPIDERMIS	

Example: Renal tubulus

Fig. 21: Proximal tubulus



Typical leaky resorptive epithelium. Na^+ is expelled by a pump at the basolateral membrane, and passively sucked up at the apical site. 2 modes of this passive motion:

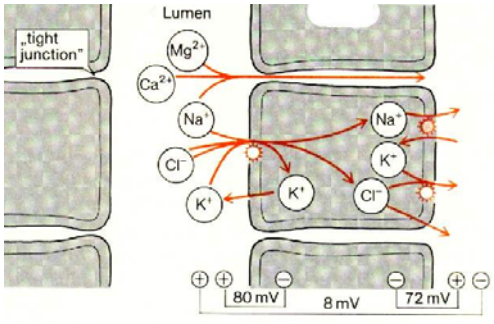
- symport with anions
- antiport with H^+

Cl^- ions are rather „neglected" owing to the prevailing transport of bicarbonate and amass in the lumen. (They should be sucked up into the cells via Na^+ /K^+ symport, but the energy of Na^+ is used here mainly for H^+.) Cl^- ions now move a whole cascade of flows:

- dc Cl^- → diffusion of Cl^- through the tight junctions into interstitium (a small dc is sufficient for dissusion on a leaky epithelium)
- escape of Cl- → electropositivity of the lumen → expelling of positive ions Na^+, Ca^{2+}, Mg^{2+} into the blood
- flow of electrolytes → osmotic flow of water → solvent drag

(Analogy in secretory epithelia where Cl^- → trans- and paracellular flow in to the lumen)

Fig. 22: Henle´s loop (ascending part):

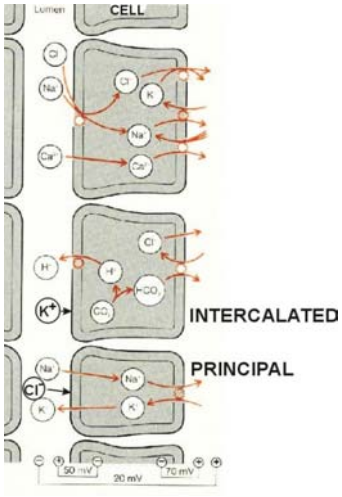


Bicarbonate: Its resorption is driven by H^+ secretion, this being driven by Na/K-ATPase or H^+-ATPase. HCO_3^- reacts with H^+ to CO_2 and water, CO_2 forms again H^+ and HCO_3^- after having penetrated into a cell, bicarbonate is then „pushed" into the interstitium. ↑ CO_2 in the blood → ↑ H^+ secretion (renal compensation of respiratory acidosis)

H^+ secretion → HCO_3^- resorption and acidification of the urine → it modulates the amount of acids excreted in a form of ammonia ions

The task: sequester water from NaCl → the epithelium must be fairly tight.
There is „correctly" NaCl/KCl symport at the luminal site. K^+ channels predominantly at the apical site → they polarize the cellular membrane more than the basolateral membrane is polarized → voltage across the whole cell → cations flow into interstitium paracellularly
The whole cell is in an electric field → the low permeability of tight junctions is overcome by it

Fig. 23: Distal nephron



Typically, the resorption is against a high gradient here → a fine „tuning" of urine is possible. Typically, the Na^+/K^+-ATPase is situated basolaterally → sucking up of Na^+ from the lumen into the interstitium
The proximal segment of the distal tubulus is similar to the Henle´s loop, only K^+ is not symported in the cell and does not return back; (sec. active) transport of Ca^{2+}. The urine is further actively diluted
Intercalated cells (type A and B): Either H^+ is secreted and HCO_3 - absorbed, or vice versa, according to the need. Important for maintaing of AB balance

Principal cells **secrete K^+:**
Na^+ is resorbed by sucking up into the principal cells; the resorption of Cl^- possibly HCO_3^- is neglected → large negative potential in the lumen → the *secretion of K^+ and H^+ is promoted*
Regulation may complicate the picture: *aldosterone* → ↑ Na/K-ATPase → ↑ K^+ and ↓Na^+ concentration in the cells → ↑ Na^+ resorption
Further, aldosterone → ↑ permability of the membrane for K^+
Trivial regulatory factors:
↑ K^+ concentration in plasma → also in the cells → easier secretion
The velocity of flow of the urine → ↓ K^+ concentration in it etc.

2. Pathophysiology of membrane transports

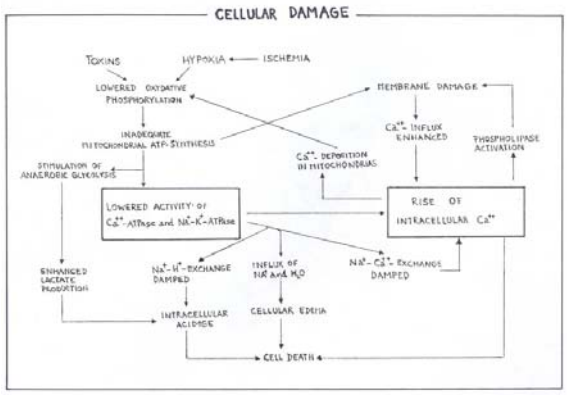
2.1 The cell

Examples of pathophysiological processes on cellular membranes

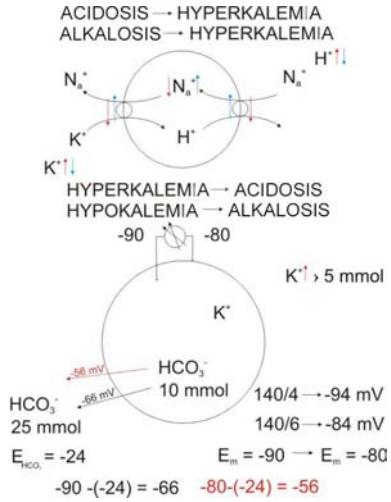
Intracellular Ca^{2+}, antiport Na/Ca
Digitalis: ↓ Na/K-ATPase → ↑intracell. Na → ↑intracell. Ca → ↑myocardial contractility
Potentitation of digitalis: hypokalemia → ↓ Na/K-ATPase
Hyperkalcemia → ↑ chemical gradient of Ca2+

Essential hypertension
(Either ↑ permeability for Na or ↓ Na/K-ATPase or ↓Ca-ATPase)
→ ↑intracell. Ca → smooth muscle contraction

Lack of energy: Fig. 24



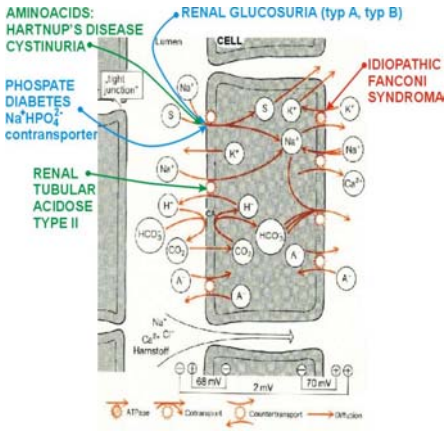
Na/H antiport, acidosis
Fig. 25: Acidosis → hyperkalemia, alkalosis → hypokalemia



2.2 Epithelia

Example: Renal tubulus

Defect of Na^+/K^+-ATPase or of its energy supply: idiopathic Fanconi syndroma
(Fig. 26)



Resorption mechanisms connected with Na^+ transport: ↑excretion of glucose, aminoacids, phosphate, HCO_3^-, uric acid

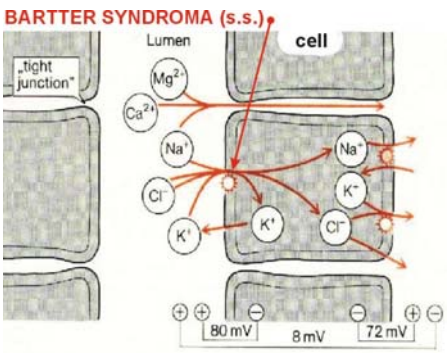
$Na^+HPO_4^{2-}$ cotransporter: inborn hypophosphatemia = phosphate diabetes = vitamin D resistent rachitis:
osteomalatia, sometimes as a component of the Fanconi syndroma

Na^+/H^+ antiport: renal tubular acidose type II:
Disturbance of the antiporter; distal nephron does not resorb large quantities of HCO_3^- → bicarbonaturia. Decline of plasmatic HCO_3^- → ↓filtration → the urine has normal pH → only ↓ plasma concentration of HCO_3^-. A possible combination with Na^+/K^+-ATPase defect (Fanconi´s syndroma).

Renal glucosuria type A:
↓number of transporter molecules → ↓maximum transport velocity (T_m)
Type B:
Disturbance of the transporter molecule → ↓substrate affinity; low affinity → resorption depends mainly on the luminal concentration of a metabolite
↑ glucose clearance → ↑glucose excretion
Osmotic diuresis

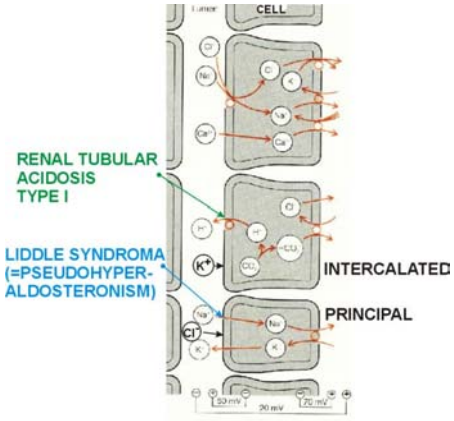
Aminoacids:
↑ plasmatic concentration of one of them → overlasting of the symport for the others → excretion of several aminoacids
Kidneys, gut and liver disturbed often at the same time→ ↓plasmatic concentration of aminoacids
„Classic" cystinuria: cystin has its own transporter
Hartnup´s disease: disturbance of the transporter for neutral aminoacids
Aminoacid disturbances may be compensated by a tertiary cotransport H^+/di(tri)peptides

$Na^+Cl^-K^+$ symport: Bartter syndrome (s.s.) (Fig. 27)



Bartter syndrome:
↑flow of Na^+ into the distal segments → ↑ Na^+ resorption in the distal tubulus → ↑ K^+ secretion. (Renal kalium loss connected with normal blood pressure = Bartter syndroma s.l.) Heavy hypokalemia. The same mechanism is active in furosemid which does not spare K^+ (pseudo-Bartter syndroma)

Na^+ channels in the distal tubulus, possibly in the colon: Liddle syndroma (= pseudohyperaldosteronism) (Fig. 28)



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Channels do not respond to aldosteron, permanently activated → ↑ K^+ and H^+ secretion, hypertension, hypokalemia, alkalosis. The channel may be blocked by amilorid

H^+/K^+-ATPase: renal tubular acidosis type I:
Inborn error of the pump, ↓acidification of the urine, ↓excretion of titratable acidity and of NH_4^+. The anions of rather strong acids must be excreted more as Na^+ a K^+ salts (i.e., of alkalic metals) → massive acidosis, the salts of Ca^{2+} a Mg^{2+} are mobilised from the skeleton

Example:

Fig. 29

CYSTIC FIBROSIS

CLINICAL TRIAS:
- Abnormally high NaCl concentration in sweat
- Inflammatory derangement of the respiratory airways
- Atrophy of exocrine pancreas

ETIOPATHOGENESIS:
Disturbance of CFTR = Cl^-channel
↓
Mucus hyposecretion ↓
↑Conductivity of a membrane of the cells of the airways for Na
↓
↑Mucus absorption in the bronchi
↓
↓Necessary volume of fluids on the surface of the airways
↓
Disturbance of cilia movement and of celea-rance of mucous membranes
↓
Bacterial colonisation
↓
Destruction of pulmonary structure

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Gene therapy of CF:

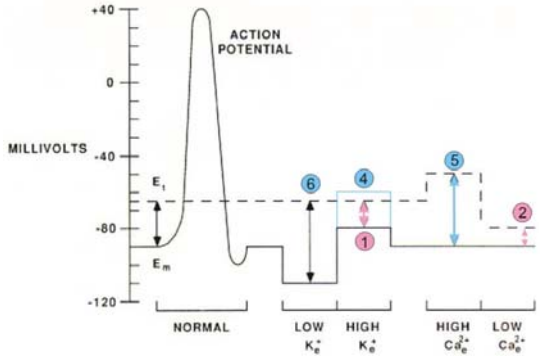
Conventional therapy (physiotherapy, antibiotics, supplementation of pancreatic enzymes)
Amilorid – Na^+ blocker

Transfer of genes:
cDNA with an appropriate promoter, bound to a gene transfer agent (GTA).
GTAs: virus, plasmid DNA, cationic liposome
Plasmid is an extrachromosomal genetic element in bacteria. A circular, double-stranded DNA molecule that usually confers some advantage to the host organism. Plasmids replicate independently of the bacterial chromosome and constitute a useful tool in recombinant DNA technology

Liposome is an arteficially prepared, cell-like structure in which bimolecular layer(s) of phospholipid enclose an aqueous compartment
Adenoviruses are used most often, their coat may stimulate immune reactions, however.
cDNA is incorporated into endosomes after a succesful transfer, it must get into the cytoplasm and the nucleus. The viruses lyse endosomes, liposomes do not
Application route: nebulisation and local application into the airways. I.v. administration?
Experimental success on primates and transgenic mice

2.3 Excitable tissues

Fig. 31 Effect of extracellular concentration of K^+ and Ca^{2+} on the rest potential E_r and the threshold potential E_t.



31

The excitability of a membrane is determined by the *difference* between the rest potential E_r and the threshold potential E_t.

Hyperkalemia, ischemia, enhanced permeability of a membrane → ↓concentration gradient of K^+ → depolarization → ↓ (E_r - E_t) → ↑excitability → convulsions (Fig. 31, 1)

↓↓ K^+ gradient → depolarization above (more positive than) Et → repolarization impossible → depolarization blockade → paralysis (4)

↑K+ gradient → hyperpolarization → ↑(E_r - E_t) → ↓excitability → paralysis (6)

The *ratio* of K^+ concentration inside and outside is important → chronic hypokalemia may lower both extra- and intracellular K^+ concentration → small effect on the potential even with low absolute values of K^+

Calcium disturbances → changes in the threshold potential
Extracellular calcium neutralizes fixed negative charges in the outer side of a membrane → it „stabilizes" membrane potential:
Hypercalcemia → ↓Na^+ permeability → E_t more positive → (E_r - E_t) more positive → ↓excitability (5)
Hypercalcemia as antidotum for hyperkalemia
Hypocalcemia → ↑Na^+ permeability → E_t more negative → (E_r - E_t) more negative → ↑excitability → tetania (2)

Lowering of extracellular pH has analogical effects as hypercalcemia

Disturbance of Cl^- channels → disturbed repolarization → ↑excitability (not in the Fig. 31)